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Original Research Article

Antibacterial activity of tekelan leaf ethanol extract (*Chromolaena odorata* (L.) R. M. King and H. Rob) against of *Staphylococcus aureus* bacteria

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ABSTRACT

Background: Indonesia is a tropical country, so the prevalence of infectious diseases caused by bacteria still remains high. On the other hand, the intense use of antibacterial agent in Indonesia causes a tendency for bacterial resistance to existing antibacterial drugs. One of the herbal plants that is widely used by some people is tekelan leaves. The purpose of this study was to determine the activity of an ethanol extract of tekelan leaves on the growth of *Staphylococcus aureus* bacteria.
Methods: Simplisia of tekelan leaves was tested for secondary metabolites and simplisa characterization testing. Antibacterial activity testing is carried out by the disc paper method, where the disc paper is immersed in the test solution with a comparison of the test concentration.
Results: The results of phytochemical screening show that the secondary metabolites of tekelan leaves are alkaloids, flavonoids, steroids/triterpenoids, tannins, and glycosides. The results of the characterization of simplisia moisture content are 7.99%, water-soluble juice content is 13.29%, ethanol-soluble juice content is 12.18%, total ash content is 7.45%, and insoluble acid ash content is 0.92%. The observation of the antibacterial activity of the ethanol extract of tekelan leaves was carried out by the disc diffusion method. The test results showed that the ethanol extract of tekelan leaves was categorized as moderate at concentrations of 1% and 2% and as strong at concentrations of 4%, 5%, 10%, 20%, 40%, 60%, 80%, and 100%. The conclusion of this study is that ethanol extract had activity to inhibit the growth of *Staphylococcus aureus* and the MIC value obtained 0.7%.
Conclusions: The conclusion of this study is that ethanol extract had activity to inhibit the growth of *Staphylococcus aureus*.

Keywords: Tekelan leaf, Phytochemical screening, Characterization, Antibacterial, *Staphylococcus aureus*

INTRODUCTION

Indonesia has natural resources in the field of herbal plants. Herbal plants have been used for generations in the community. This alternative is commonly practiced in rural communities because the distance between the residence and the location of health services is limited and even difficult to reach. Until now, it is still widely used in rural areas and is also still being researched and developed; moreover, herbal medicines have fewer side effects compared to synthetic drugs provided in health care facilities.[1]

One herbal plant that is widely used by some people is tekelan leaves. However, tekelan leaves are still widely ignored by the community and only used as weeds.

Tekelan leaves have many benefits, such as being anti-inflammatory, antibacterial, antidiarrheal, antioxidant, and antidiabetic. Some previous studies have tested antibacterial activity using tekelan leaves against *Salmonella typhi, Shigella dysenteriae, Staphylococcus epidermidis*.[2,3]

The intense use of antibiotics as antibacterial agent in Indonesia has led to the development of bacterial resistance to existing antibiotic drugs. This condition is caused by a variety of factors, ranging from the patient's health condition, drug use factors, and the microorganism factor itself.[4] Because of the side effects of chemical drug use, it is therefore necessary to develop the use of traditional medicine to reduce the side effects that may occur in patients.

Based on the background, the researchers are interested in testing the inhibitory activity of the ethanol extract of tekelan leaves because of its metabolite content, which stops bacterial growth. They also want to determine the minimum inhibitory concentration (MIC) against *Staphylococcus aureus*.

METHODS

This study was conducted from February to April 2023 in the laboratory of Phytochemistry and Microbiology, Faculty of Pharmacy, Universitas Sumatra Utara, Medan, Indonesia.

Tools

The tools used in this study are beakers, measuring cups, funnels, test tubes, test tube racks, dropper pipettes, micro pipettes, porcelain cups, hot plates, vials, glass objects, glass stirring rods, spatulas, porcelain cups, filter paper, analytical balance (GR-200), water bath, digital calipers, scissors, incubator (Memmert), oven (UN-55), and autoclave.

Materials

The materials used in this study were tekelan leaves taken from Deli Serdang, hydrochloric acid reagent (HCl), ammonia, chloroform, magnesium, distilled water, sulfuric acid (H_2SO_4), Mayer reagent, Dragendorff, Bouchardat, $FeCl_3$ 10%, DMSO, *Mueller Hinton* agar (Merck), bacterial suspensions, 70% ethanol, plastic wrap, aluminum foil, and parchment paper.

Preparation of tekelan leaf simplisia powder

Simplisia powder is made from tekelan leaves that have been dried for 2 to 3 days at temperatures below 40 °C with a tool without causing damage or loss of the required chemical content and sieved until a fine powder is obtained. The degree of fineness of the simplisia powder consists of very coarse, coarse, somewhat coarse, fine, and very fine powder.[4]

Preparation of ethanol extract of tekelan leaf

Preparation of ethanol extract from tekelan leaves using the maceration method with a ratio of 1:10. 1 part of tekelan leaf simplisia powder was put into a glass container, and 10 parts of 70% ethanol solvent were added. Soaked for 3 days while occasionally stirring, then allowed to stand. Then filtered. The process was repeated once over a period of 2 days, and then all the maserat was collected and evaporated with a rotary evaporator until a thick extract was obtained.[5,6]

Phytochemical screening

Simplisia and ethanol extracts of tekelan leaves underwent phytochemical screening, which included steroid/ triterpenoid analysis, alkaloid analysis, glycoside analysis, saponin analysis, flavonoid analysis, and tannin analysis.[7]

Identification of alkaloid

The simplisia powder is combined with distilled water and HCl_2N, boiled on a water bath, and then filtered. The filtrate is taken three times and placed in a test tube, where the Mayer reagent, Dragendorff, Bouchardat reagents are added. If there is a precipitate on two of the three reagents, the alkaloids are considered positive.[8]

Identification of saponin

The simplisia powder is put in a test tube, hot water is added, and it is shaken vigorously for 10 seconds. A good froth is formed for at least 10 minutes, as high as 1–10 cm, and does not disappear with the addition of HCl_2N.[9]

Identification of tannin

The simplisia powder is macerated with distilled water for 15 minutes, then filtered. After 15 minutes, diluting the filtrate until it becomes colorless, two drops of a 10% $FeCl_3$ solution are added. It is said to be positive for tannin if there is a blue or green color in the filtrate.[10]

Identification of flavonoids

The powdered simplisia was dissolved in hot water, brought to a boil, and filtered while hot. Taken filtrate is added with magnesium powder and a concentrated HCl solution of mil alcohol funds. It is said to be positive for flavonoids if a red or orange-yellow color occurs in the amyl alcohol layer.[11]

Identification of steroid/triterpenoids

The simplisia powder was macerated with an N-Hexan solution and filtered. The filtrate is evaporated, and the rest is added with Liebermann-Bouchard reagent through the cup wall. It is said to be positive for triterpenoids or steroids if a red color is formed that turns blue-green.[12,13]

Identification of glycosides

The simplisia powder was dissolved in an ethanol solvent, evaporated in anhydrous acetic acid, and then slowly added a little concentrated H_2SO_4 through the test tube wall. It is said to be positive for glycosides if a blue or green color is formed in the filtrate.[14]

Characterization of tekelan leaf simplisia

Determination of moisture content

The determination of moisture content was carried out according to the azeotropy method (toluene distillation). The apparatus consisted of a 500-ml round-bottom flask, cooler, connecting tube, 5 ml receiving tube with a 0.05 ml scale, collection device, and electric heater.

Determination of water soluble essence content

The sample was put in a corked flask with 100 ml of chloroform saturated water, shaken for the first 6 hours, and let stand for 18 hours. Filter, then evaporate. 20 ml of filtrate is taken and put in a flat bottom flask that has been heated at 105 °C and distilled. Calculate the content of water-soluble essence in percent.

Determination of ethanol soluble juice content

The sample was put in a corked flask with 100 ml of 96% ethanol, shaken for the first 6 hours, and let stand for 18 hours. Filter, then evaporate. 20 ml of filtrate was taken and put in a flat bottom flask that had been heated at 105 °C and distilled. Calculate the water-soluble essence content in percent.

Determination of total ash content

The pulverized sample is placed in a silicate chair that has been incinerated and tarred in a device called a furnace, heated at 600 °C. Total ash content is calculated by the weight of the test material expressed in % b/b.

Determination of acid insoluble ash content

Samples that have become ash are heated with HCl for 5 minutes. Then it was filtered through ash-free filter paper, washed with hot water, and incinerated in a furnace at 600 °C. Acid insoluble ash content is calculated by the weight of the test material expressed in % b/b.

Testing antibacterial activity of tekelan leaf against Staphylococcus aureus

Sterile *Mueller Hinton* agar (MHA) media was put 20 ml into sterile petri dishes. Each petri dish containing MHA was leveled and allowed to solidify. Then, enter the concentration of ethanol leaf extract tekelan positive control and negative controls using a micropipette and place them on a sterile paper disc. The disc paper was placed into the petri dish and left for a while so that the diffusion process could take place. Next, the petri dish was incubated in an incubator at 37°C for 24 hours. After incubation, calculate the diameter of the inhibition zone with a digital caliper. This was done for three repetitions. This was done to ensure that all inhibition zones in each experiment were obtained under the same experimental conditions.[15,16]

Observation and measurement of inhibition by disc method

Observations were made after 1×24 hours of incubation in an incubator. The area around the disk appears to have microbial sensitivity to antibiotics or antimicrobial materials used as test materials, as expressed by the diameter of the inhibition zone in the clear zone formed. The diameter of the inhibition zone is measured in millimeters (mm) using a digital caliper by measuring the distance from the edge of the test to the circular boundary of the inhibition zone/clear zone. Then the diameter of the inhibition zone is categorized by the strength of the antibacterial power based on the classification, whether it is strong (>20 mm), moderate (>10–20 mm), or weak (<10 mm).

The MIC of tekelan leaves of ethanol extract is obtained based on the following equation.

$$ln(MIC) = In(c)\frac{x^2}{4Dt}$$

MIC is minimum inhibitory concentration, x^2 as zone of inhibition (mm), c as the concentration (%), D as diffusion coefficient, and t as time required for diffusion.[17]

RESULTS

Phytochemical screening of ethanol extract of tekelan leaf

The content of secondary metabolites in the ethanol extract of tekelan leaves has been tested qualitatively, showing the presence of secondary metabolite content including alkaloids, flavonoids, steroids, saponins, tannins, and glycosides. The test results can be seen in the following Table 1.

Table 1: Secondary metabolite compounds of tekelan leaf simplisia.

Secondary metabolite	Result
Alkaloids	+
Flavonoids	+
Steroid/triterpenoids	+
Saponins	+
Tannins	+
Glycosides	+

The results were identified by the findings of the analysis of secondary metabolite chemicals. This group is identified by the production of a white precipitate on Meyer's reagent, a reddish-brown precipitate on Wagner's reagent, and Dragendorff.[7] The three reagents showed positive results, where alkaloids are declared positive when 2 or 3 perekasi showed positive results. Examination using $FeCl_3$ on the ethanol extract of tekelan leaves showed positive results, namely color changes into strong green, red, purple, and black colors.[18] This indicates that the ethanol extract of tekelan leaves is positive for tannin compounds. Saponin examination by shaking with hot water and adding HCl_2N shows stable foam formation, so the ethanol extract of tekelan leaves also contains saponin compounds.[19]

Flavonoid examination showed positive results, also marked by a change in color that occurs that is reddish black, so this is declared positive containing flavonoids.[20] The reaction using the Lieberman-Bouchard reagent gives a red-purple color (showing positive triterpenoids), while if it shows a green-blue color (positive triterpenoids).[21] Samples showed positive results on steroids and triterpenoids.

Characterization of tekelan leaf (Chromolaenaodorata (L.) R. M. King and H. Rob)

The results of the examination of the characteristics of tekelan leaf simplisia, which include examination of water content, water soluble juice content, ethanol soluble juice content, total ash content, and acid insoluble ash content, meet the requirements in Materia Medika Indonesia, as shown in Table 2.

Table 2: Characteristics of tekelan leaf simplisia.

Parameter	Result (%)	Materia Medika Indonesia (%)
Water content	7.99	<10
Water soluble essence content	13.29	>6.4
Ethanol soluble essence	12.18	>8
Total ash content	7.45	<7.6
Insoluble ash content	0.92	<1

Analysis of water content was carried out by the azeotropy method (toluene distillation). The data generated from the analysis of simplisia water content shows the water content in tekalan leaves is 7.99% (b/b). The maximum moisture content required for the extraction process is 10%, so it can be seen that the water content in the tekelan leaf samples does not exceed the predetermined maximum limit.

Determination of the content of soluble compounds in water and ethanol solvents aims to estimate the content of active compounds that are polar (soluble in water) and are polar - non-polar (soluble in ethanol).[22] In tekalan leaf simplisia, the content of compounds soluble in water and ethanol solvents is 13.29% and 12.18% respectively. The results obtained indicate that the compounds from tekelan leaves are more soluble in water than ethanol.

The determination of total ash content is carried out to provide an overview of the internal and external mineral content from the initial process until the formation of the extract. The total ash content in tekelan leaf simplisia was 7.45%. The higher the ash content obtained, the higher the mineral content in the material. Minerals are needed by humans, such as calcium, phosphorus and magnesium for bone growth. Sodium and chloride for body fluids, iron for the formation of haemoglobin and red blood cells. This is not the case with toxic minerals (metals). This is not the case with toxic minerals (heavy metals) such as mercury, lead, copper, cadmium and strontium. Accumulation of heavy metals in the human body over a long period of time can disrupt the circulatory system and the work of the kidneys. Insoluble acid ash content reflects the presence of mineral contamination or insoluble-acid metals in a product. Insoluble acid ash content in tekelan leaf simplisia was 0.92%. The high acid insoluble ash content indicates the presence of silicates derived from soil or sand, soil and metal elements of silver, lead and mercury.[23]

Antibacterial activity of tekelan leaves of ethanol extract (Chromolaena odorata (L.) R. M. King and H. Rob)

The results of the activity test of the ethanol extract of tekelan leaves against *Staphylococcus aureus* bacteria with test concentrations of 1; 2; 4; 5; 10; 20; 40; 60; 80; and 100% to measure the inhibition zone in the form of clear zones produced can be seen in Table 3.

Table 3: Antibacterial activity of tekelan leaf of ethanol extract.

Concentration (c) TLEE	ln (c)	Average diameter of inhibitory (x)±SD	x^2
0	0	0	0
1	0	2.13±0.04	4.53
2	0.6931	3.23±0.04	10.43
4	1.386	5.37±0.04	28.72
5	1.7917	5.87±0.04	33.98
10	2.3025	6.76±0.04	45.69
20	2.996	7.57±0.04	57.15
40	3.689	8.73±0.04	76.21
60	4.094	9.37±0.04	87.6
80	4.382	9.97±0.05	99.2
100	4.605	10.93±0.05	119.46

Antibacterial activity is classified as weak when the inhibition zone is less than 5 mm, as moderate in the inhibition zone of 5–10 mm, as strong when the inhibition zone is 10–20 mm, and as very strong in the inhibition zone above 20 mm.

Determination of MIC of tekelan leaf of ethanol extract using graphs

Equation (1) is then derived into the following where MIC is minimum inhibitory concentration, X is diameter of inhibition, D is diffusion coefficient, and t is diffusion time.

$$ln(c) = In(MIC)\frac{x^2}{4Dt}$$

Determination of the MIC value using a graph is to make the intercept equation of the In(c) plot value with x.[17] The MIC graph for TLEE can be seen in Figure 1.

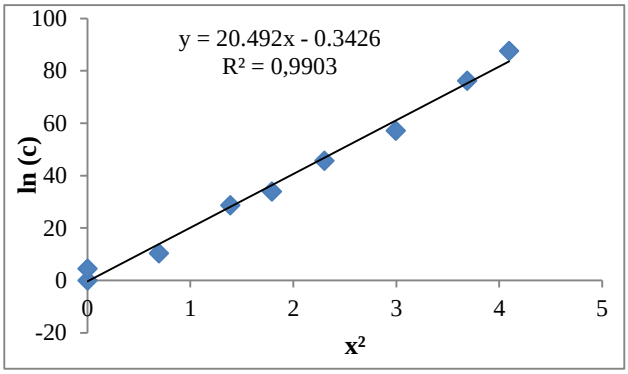

Figure 1: Plot of ln(c) versus x² of ethanol extract of tekelan leaves against *Staphylococcus aureus.*

From Figure 1, it can be seen that the line equation obtained has a correlation coefficient of 0.9903. This shows a good linear relationship. The intercept value of the line equation is -0.3426. The MIC value obtained is 0.7% with the calculation of anti-ln (c) versus x^2.

DISCUSSION

The content of secondary metabolites in tekelan leaves of ethanol extract has been tested qualitatively showing the content of secondary metabolites including alkaloids, flavonoids, steroids, saponins, tannins and glycosides.

The characterisation of simplisia carried out includes water content, total ash content, water soluble juice content, ethanol soluble juice content, acid insoluble juice content. Determination of simplisia characteristics in this test meets the specified requirements.

The test results show that the tekelan leaves of ethanol extract is included in the moderate category at concentrations of 1% and 2% and is classified as strong at concentrations of 4%, 5%, 10%, 20%, 40%, 60%, 80%, and 100% inhibition. *Staphylococcus aureus* bacteria with a polar peptidoglycan layer, so the flavonoids contained in the extract can penetrate the peptidoglycan layer rather than the lipid layer, which is non-polar. Other metabolite compounds in the form of tannins can also inhibit bacterial growth by coagulating bacterial protoplasm. *Staphylococcus aureus* bacteria have a polar peptidoglycan layer; the extract's flavonoids can pass through it instead of the non-polar lipid layer. By coagulating bacterial protoplasm, other metabolite chemicals, such as tannins, can also prevent the growth of bacteria.[21]

In this study, the minimum inhibitory concentration of the extract in inhibiting bacterial growth was determined. However, the minimum inhibitory concentration value was only tested on *Staphylococcus aureus* bacteria.

CONCLUSION

Phytochemical screening of simplisia and ethanol extracts of tekelan leaves showed good results. They contained secondary metabolite compounds of alkaloid, flavonoid, tannin, saponin, glycoside, steroid/triterpenoid groups, and had the ability to stop *Staphylococcus aureus* bacteria from growing.

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